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Antagonism of *Trichoderma* spp. strains against pea (*Pisum sativum* L.) Fusarium wilt caused by *Fusarium oxysporum* f. sp. pisi.

Antagonismo de cepas de *Trichoderma* spp. sobre el marchitamiento de arveja (*Pisum sativum* L.) causado por *Fusarium oxysporum* f. sp. pisi.

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Abstract

The antagonistic effectiveness of native strains of *Trichoderma* spp. on *Fusarium oxysporum* f. sp. pisi. *in vitro*, greenhouse and field conditions, were evaluated. *in vitro* conditions, the antagonistic capacity of 12 strains of *Trichoderma* spp., C2, C7, C12 and C21 strains, exhibited a better behavior measured by the following variables: inhibition halo and mycelial growth. In greenhouse conditions, the four strains, which showed the best *in vitro* antagonistic behavior, were evaluated using a DIA experimental design with factorial arrangement for three factors, which corresponded to strain, concentration and dose. The results of this evaluation, showed that C12 and C21 strains at doses of 20 mL, and at concentrations of 10^8 and 10^6 conidia.mL⁻¹, respectively. The best antagonistic response was determined by variables as follows: plant height, fresh root weight and incidence. Under field conditions, the evaluations were carried out in the municipalities of Ipiales, Pupiales and Gualmatán, in the department of Nariño, Colombia. In each location, a BCA experimental design was used with four treatments and five replicates, treatments were as follows: C12 strains at 10^8 concentration, C21 at 10^6 concentration, chemical control and absolute control. In Gualmatán location, C12 and C21 strains, showed no antagonistic capacity, whereas in Ipiales and Pupiales locations, strain C12, presented a lower incidence of *F. oxysporum* than the control, but with no effect on yields. In Pupiales location, C21 strain surpassed in performance to the control treatment, even though the two treatments had similar incidence.

Key words: Antagonistic capacity, inhibition halo, inoculum, *in vitro*, field conditions, greenhouse, mycelial growth.

Resumen

Se evaluó la efectividad antagónica de cepas nativas de *Trichoderma* spp. sobre *Fusarium oxysporum* f. sp. pisi. en condiciones de laboratorio, invernadero y campo. En condiciones de laboratorio, se evaluó la capacidad antagónica de 12 cepas de *Trichoderma* spp., las cepas C2, C7, C12 y C21, mostraron un mejor comportamiento medido por las variables: halo de inhibición y crecimiento micelial. En condiciones de invernadero, se evaluaron las cuatro cepas que evidenciaron mejor comportamiento antagónico *in vitro*, utilizando un diseño experimental DIA con arreglo factorial para tres factores que correspondieron a cepa, concentración y dosis. Los resultados de esta evaluación mostraron que las cepas C12 y C21 en dosis de 20 mL, y en concentraciones de 10^8 y 10^6 conidias.mL⁻¹, respectivamente. Se obtuvo mejor respuesta antagónica determinada por las variables: altura de planta, peso fresco de raíces e incidencia. En condiciones de campo, las evaluaciones se realizaron en los municipios de Ipiales, Pupiales y Gualmatán, en el Departamento de Nariño, Colombia. En cada localidad, se utilizó un diseño BCA para cuatro tratamientos y cinco repeticiones, los tratamientos fueron las cepas C12 en concentración 10^8 , C21 en concentración 10^6 , control químico y testigo absoluto. En la localidad de Gualmatán, las Cepas C12 y C21, no mostraron capacidad antagónica mientras que en Ipiales y Pupiales, la cepa C12 presentó menor incidencia de *F. oxysporum* que el testigo, pero sin efecto sobre los rendimientos. En la localidad de Pupiales, C21 superó en rendimiento al testigo absoluto aun cuando los dos tratamientos presentaron similar incidencia.

Palabras clave: Capacidad antagónica, condiciones en campo, crecimiento micelial, halo de inhibición, inóculo, *in vitro*, invernadero.

Introduction

Pea wilt (*Pisum sativum* L.) caused by *Fusarium oxysporum* f. sp. *pisi*. is one of the prevalent diseases in intensive crops and can cause losses ranging from 30 to 50%, especially in susceptible varieties and under favorable climatic conditions for the pathogen development. The disease symptoms starts with an ascending chlorosis, followed by plant stunting, in the outside of roots, a brown coloration is seen and in the inner part, a longitudinal lesion of reddish tonality. The fungus elicits a premature death by plant wilt associated to redness and blockage of vascular bundles (Buitrago *et al.*, 2006; FENALCE, 2007).

Due to yellowing and wilt of pea, this disease can occur for different causes. The real test to pathogen susceptibility, it would be due to vascular bundles redness in the root, which produces the other two symptoms as side effects.

In Nariño department, Colombia there are no resistant varieties and the efforts of farmers to control this problem are not satisfactory because the fungus is still widely distributed and persistent in soils (Sañudo *et al.*, 2007).

There are different studies of the genus *Trichoderma* spp. in the biological control of soil pathogens transmitting diseases in plants whose results indicates that several strains of *Trichoderma* show a significant reductive effect in plants with diseases caused by pathogens such as *Rhizoctonia solani*, *Sclerotium rolfsii*, *Pythium aphanidermatum*, *Fusarium oxysporum*, *F. culmorum*., under greenhouse and field conditions. Accordingly, to the harmful behavior of these fungi, the use of antagonists represents an effective and essential use, since they can act against the pathogens in several ways, isolates of *Trichoderma harzianum*, can produce lytic enzymes antibiotics and antifungals. In addition, they can be efficient pathogenic fungi competitors and to promote plant optimal growth (Gajera *et al.*, 2013).

The aim of the present research was to evaluate the antagonistic effectiveness of *Trichoderma* spp. native and commercial strains on *Fusarium oxysporum* f. sp. *pisi*., under *in vitro*, greenhouse and field conditions.

Materials and methods

Bioassays were carried out in the Universidad de Nariño, Colombia. In the plant health laboratory, at a temperature of 13 °C, under greenhouse conditions, 17 °C, and an altitude of 2540 m. a. s. l., with the following geographical coordinates: 01°12'13"N; 77°15'23"W. The field phase was established in the most affected disease area in the Nariño department, Colombia

corresponding to the municipalities of Pupiales (2875 m.a.s.l.), Ipiales (2665 m.a.s.l.) and Gualmatán (2714 m.a.s.l.), located respectively and geographically as follows: between 0° 52' N and 77° 38' W, 0°59'39"N, 77°33' 56"W, and 0°50' N and 77°34' W, with a temperature which oscillates between 11 and 12 °C, and an annual rainfall between 500 and 1100 mm, which varies according to the time of year.

To obtain the different *Trichoderma* spp. native strains, 20 representative soil samples were collected from each of the evaluated municipalities, taken from the soil rhizosphere of apparently healthy pea plants. For pathogen isolation, pea plants with yellowing disease symptoms, ascending chlorosis, low growth and reddish coloration in vascular bundles, were considered. *Trichoderma* spp. isolates were performed by the dilution method, weighing 1 g of collected soil and placing it in a test tube with 10 mL of distilled water. The process was brought to a 10⁻² dilution, and then, seeded into PDA with chloramphenicol. *Trichoderma* spp. colonies with characteristic growth form of green cushions of conidia, were identified and under microscope, the genus characteristics, were confirmed (Barnett & Hunter, 1972). Twelve strains of *Trichoderma* spp. from the municipalities of Pupiales, Ipiales and Gualmatán, were obtained. The obtained *Trichoderma* spp. strains in addition to commercial control strain, identified as C21 from Perkins laboratories located at Palmira – Valle del Cauca, Colombia, constitute a group of 13 treatments which *in vitro* antagonism tests, were performed. In the 12 strains of *Trichoderma* spp., the species were identified in a simultaneous research, determining the presence of *T. harzianum*., *T. longibrachiatum* and *T. asperellum* (Table 1), Being the most frequent, *T. harzianum* (Checa *et al.*, 2015).

Table 1. *Trichoderma* spp. strains collection in Nariño department, Colombia

Strain	Species	Location
C1	<i>T. harzianum</i>	Pupiales
C2	<i>T. harzianum</i>	Pupiales
C3	<i>T. asperellum</i>	Pupiales
C4	<i>T. asperellum</i>	Pupiales
C5	<i>T. harzianum</i>	Gualmatán
C6	<i>T. longibrachiatum</i>	Gualmatán
C7	<i>T. harzianum</i>	Gualmatán
C8	<i>T. harzianum</i>	Gualmatán
C9	<i>T. longibrachiatum</i>	Ipiales
C10	<i>T. harzianum</i>	Ipiales
C11	<i>T. harzianum</i>	Ipiales
C12	<i>T. harzianum</i>	Ipiales
C21	<i>T. harzianum</i>	Perkins

For each location, the isolation of *Fusarium oxysporum* f.sp. pisi was established from the affected pea plant material by taking root tissue and symptomatic stems under aseptic conditions. Sections were seeded in PDA and incubated at room temperature until the development of fungal colonies (Rojas, 2011), from the colony edge, monosporic cultures were generated, until a pure colony was obtained, which served as base for the fungi multiplication.

in vitro antagonism assay

An unrestricted random design was performed with 13 treatments corresponding to the strains of *Trichoderma* spp., in addition to one commercial strain and three replicates, the experimental unit being a petri dish. The methodology described by Howell (2003), was used, placing on a side of a petri dish with PDA a 5 mm diameter disk of *Fusarium oxysporum* f.sp. pisi culture, during the time necessary for this to initiate mycelial growth. One day later, a culture of 5 mm diameter disk of each *Trichoderma* spp. Native strain, was placed on the opposite side. Disk pairs have allowed incubating until evaluation of mycelial growth and inhibition halo.

Greenhouse antagonism assay

In order to identify the most appropriate strains, concentrations and doses to be evaluated in field test, were performed. We used a DIA experimental design with factorial arrangement for three factors, the first factor corresponded to the best *in vitro* behavior of following strains: C2, C7, C12 and C21. The second factor was the concentration, which had four levels as follows: 10^6 , 10^7 , 10^8 and 10^9 conidia of *Trichoderma*. mL^{-1} . The third factor was the dose to be applied with two levels: 20 and 40 mL per plant, for 32 treatments, with 2 replicates. The experimental unit corresponded to five pea plants grown in bags with 3 kg of sterile soil. Different concentrations were obtained from Petri dishes with abundant growth of *Fusarium oxysporum* and *Trichoderma* spp., removing the conidia with a handle, making serial dilutions. For *F. oxysporum*, the concentration 10^6 was used and for the case of *Trichoderma* spp., The 10^6 , 10^7 , 10^8 and 10^9 concentrations were obtained by counting conidia in the Neubauer chamber. The formula: $V1 = C2 \ V2$, where: C1 = initial concentration (known in the count), V1 = initial volume (arbitrarily established when preparing the inoculum), C2 = desired final concentration (accordingly to the study to be carried out) V2 = Final volume (unknown).

The inoculation of *F. oxysporum*, was established 8 days before sowing. Each treatment was applied in drench way in wet soil conditions. The

inoculations of *Trichoderma* spp., were performed when pea plants had a height of 10 cm. The evaluated variables corresponded to plant height at 60 days, fresh weight of roots and incidence. For the incidence, the number of pea plants with symptoms in the root system was counted, and the pathogen re-isolation was performed to assure the disease attack was caused by *F. oxysporum*.

Field phase evaluation

Bioassays were established in the municipalities of Pupiales and Gualmatan, in lots highly affected by the pathogen. With losses exceeding 70% in the previous half year. Seeding was carried out in the rainy season of the first half of 2014, using the Andean improved variety of fickle growth habit with good adaptation to the region, but susceptible to the pathogen. A randomized complete block design with four treatments and five replicates was performed at each location. The treatments corresponded to the two strains of better behavior in greenhouse with the concentration of best response obtained for each of them, being these C12 and C21, in concentration of 1×10^8 and 1×10^6 conidia. mL^{-1} , respectively. In addition, a chemical treatment of seed disinfection with oxycarboxin plus captan, was included in doses of 1g of pc per kg of seed and an absolute control. The experimental unit was two rows of five meters long, with distance among rows of 1.2 m and distance among sites of 0.10 m, depositing one seed per site. Agronomical management of pea crop was carried out using a tillage system, of common occurrence in the region and applying the cultivation technology for the zone recommended by Sañudo *et al.* (2007). The evaluated variables were as follows: incidence percentage, evaluated by the presence of reddish color in the root and yield in green pod ($\text{Kg} \cdot \text{ha}^{-1}$). The data were submitted to variance analysis and Tukey's means comparison.

Results and discussion

in vitro antagonism assay

Mycelial growth and inhibition halo were different among strains. Isolates C12, C7, C2 and C21, showed the best behavior, presenting a mycelial growth, which varied between 4.73 and 3.72 cm, exceeding C11, C5, C9, C6, C4 and C10, with averages below 3.40 cm (Table 2).

Table 2. Tukey's averages comparison for mycelial growth and inhibition halo variables of *Trichoderma* spp, in dual confrontations with *Fusarium oxysporum* f.sp. *pisi* *in vitro* condition evaluations

Strain	Mycelial growth	Strain	Inhibition halo
C12	4.73 A	C12	4.55 A
C7	4.62 AB	C7	4.33 AB
C2	4.46 AB	C2	4.33 AB
C21	4.37 ABC	C21	4.00 ABC
C1	3.72 BCD	C3	3.44 BCD
C8	3.49 CD	C11	3.33 CD
C3	3.46 CD	C9	3.22 CD
C11	3.38 D	C6	3.22 CD
C5	3.37 D	C1	3.22 CD
C9	3.36 D	C8	3.22 CD
C6	3.26 D	C4	3.11 CD
C4	3.24 D	C5	3.99 D
C10	3.18 D	C10	3.77 D

Tukey comparator α 0.05 = 0.934. Averages with the same letter do not show significant differences ($p \leq 0.05$)

It is important to note that the pathogen growth was lower when it were exposed to the strains mycelium of C2, C7, C12 and C21 *Trichoderma* spp., and it is at this point, when the antagonist capacity to overgrow the pathogen was variable for each strain. Therefore, a differential effect evidence of the strains treatments on *Fusarium oxysporum* f.sp. *pisi*.

In accordance with Gajera *et al.* (2013), the greater antagonistic capacity observed in the abovementioned strains could be related to the greater efficiency in nutrient competition or the products release, which slow or kill the *F. oxysporum* pathogen. Given these concerns, a greater mycoparasitism effect is possible. In this regard, Howell (2003), indicates the way in which *Trichoderma* spp. inhibits the pathogen growth is mediated by several mechanisms, including antibiosis, which can also be associated with the extracellular enzymes production as follows: peptinases, cutinases, glucanases, and chitinases, and inactivation of them towards the pathogen, as suggested by Durán *et al.* (2003). This information is useful in to keep in mind that *in vitro* tests are used to determine the antagonistic potential and to establish the biocontroller inhibitory capacity before carrying out studies, which require more time and economic cost (Larralde *et al.*, 2008). Respectively, the *in vitro* evaluation results of C2, C7, C12 and C21 strains were selected to perform greenhouse studies and confirm their effectiveness.

Greenhouse antagonism assay

Plant height

According to the variance analysis, the triple strain interaction * concentration * dose, was significant indicating the height results were affected by all three factors together. Therefore, only this interaction was analyzed to define within each strain and in each concentration, the behavior of the two evaluated doses. In C2 and C7 strains, no significant differences were observed between the 20 and 40 mL doses within each *Trichoderma* inoculum concentrations (10^6 , 10^7 , 10^8 and 10^9). In strain 12, a 5% of obtained probability difference between the dose of 20 ml with 146.7 cm and the dose of 40 ml, which had a height of 134.8 cm. The abovementioned in a concentration of 10^7 . C21 strain for 10^8 concentration in *Trichoderma* conidias.ml⁻¹ was obtained a higher average height in the dose of 40 ml with 154 cm, showing significant differences on the dose of 20 mL, which reached 140 cm (Table 3).

Table 3. Tukey's averages comparison for *Trichoderma* spp, strain * concentration * dose interaction in the evaluation of their antagonism to *Fusarium oxysporum* f.sp. *pisi* under greenhouse conditions.

Strain	Dose (mL)	Concentration (conidia.mL ⁻¹)			
		10^9	10^8	10^7	10^6
2	20	135.6 A	140.1 A	127.9 A	136.8 A
	40	136.5 A	140.1 A	136.4 A	133 A
7	20	121.9 A	116.9 A	113.2 A	116.2 A
	40	123.6 A	118.2 A	115 A	114.9 A
12	20	154.2 A	155.7 A	146.7 A	127.1 A
	40	155.7 A	156 A	134.8 B	123.9 A
21	20	139.3 A	140 A	142.7 A	142.3 A
	40	147.2 A	154.1 B	143.5 A	143.9 A

Tukey comparator (α 0.05) = 5.84g

Except for these two exceptions, the increase of the dose of 20 to 40 mL for the different evaluated concentrations within each strain performed an improvement in its antagonistic capacity measured by plant height at 60 days. However, the possibility dose of 40 mL was used in field conditions for C2 strain, was conditioned to obtain the same response in the fresh weight of roots and incidence variables, which did not occur since the triple interaction was not significant for these variables.

Root fresh weight

It is important to note there were no significant differences among C2, C7 and C21 strain concentrations (Table 4), whereas in the C12 strain, the 10^8 and 10^9 concentrations with

averages of 4.36 g and 4.49 g, showed significant differences on the 10^6 concentration, which reached an average of 4.03 g. With the exception of C12 strain, 10^6 , 10^7 , 10^8 and 10^9 concentrations, had similar behavior in each of the strains under evaluation, suggesting that it is not necessary for these three strains (C2, C7 and C21) to increase the *Trichoderma* concentration beyond 10^6 conidia.mL⁻¹.

Table 4. Tukey's averages comparison for fresh weight variable in the evaluation of four strains and concentrations of *Trichoderma* spp. for its antagonism to *Fusarium oxysporum* f.sp. pisi under greenhouse conditions

	Concentrations		Strains	
	C2	C7	C12	C21
10^9	3.96 A	3.82 A	4.49 A	4.32 A
10^8	3.95 A	3.71 A	4.36 A	4.06 A
10^7	3.90 A	3.71 A	4.10 B	4.08 A
10^6	3.75 A	3.54 A	4.03 B	4.11 A

These results confirm Lo *et al.* (1994), who found the application of *Trichoderma* to 10^6 concentration is effective for the control of a good number of soil fungi. However, in the case of C12 in the present research, 10^8 and 10^9 concentrations, improved their antagonistic capacity, measured throughout fresh root weight.

Incidence

In the incidence variable, differences were only found for comparison between strains. The highest incidence occurred in the control treatment with *Fusarium* and without *Trichoderma* inoculation with 100% of affected plants (Table 5).

Table 5. Tukey's averages comparison for incidence percentage variable in the evaluation of four *Trichoderma* spp. strains for its antagonism to *Fusarium oxysporum* f.sp. pisi under greenhouse conditions

Strain	Average
C7	75.05 A
C2	54.72 B
C21	34.47 C
C12	32.82 C

Tukey comparator (α 0.05) = 6.78

Similarly, the incidence was higher for C7 and C2 strains (97 and 70%), while C21 and C12 strains, showed better antagonistic behavior with a pathogen incidence of 50 and 45%, respectively.

In concordance to Woo *et al.* (2006), in addition to the antagonistic effect of *Trichoderma* strains against the invasive pathogen, associations of root fungi also stimulate plant defense mechanisms, leading to the resistance mechanisms induc-

tion similar to the hypersensitive response (RH), Systemic resistance (SR) and systemic resistance induction (SRI). At molecular level, resistance results in an increased metabolites and enzymes concentration related to defense mechanisms, such as phenylalanine ammonium lyase (PAL) and chalcone synthase (CHS), involved in the response biosynthesis of phytoalexins, chitinases and Glucanases. As well as resistance to hostile abiotic conditions. In barley, *Trichoderma atroviride*, showed an increased resistance to *Fusarium* infection *Trichoderma harzianum*, in transgenic lines of tobacco and potato plants, were highly tolerant or completely resistant to aerial pathogens as follows: *Alternaria alternata*, *Alternaria solani*, and *Botrytis cinerea*. Alternatively, *Rhizoctonia solani* in soil (Woo *et al.*, 2006).

Field antagonism assay

Differences were observed between locations and locations * strain interaction for incidence percentage and yield variables. In Gualmatan location, the incidence rate varied between 85.78 and 89.99%, with no differences between the evaluated treatments. In Ipiales location, C12 strain and chemical control (83.89 and 76.35) showed lower incidence than C21 and treatment without control (88.04 and 89.9) (Table 6). In Pupiales location, C12 strain with 80% of incidence was less than the uncontrolled treatment (100%), while the C21 strain with 89% and chemical control with 91% of incidence, showed no difference among them, neither with C12 treatments and without control. Likewise, the pathogen incidence was high in all treatments in three locations (>76%). Although the plant percentage with presence of the pathogen (incidence) was high, root redness vascular was not the same in all pea plants, there were plants with a slight presence of *Fusarium* in the root, which were productive and is possible when fungus does not severely affect the xylem vascular bundles. It is important to highlight the behavior of C12 strain C12 in Pupiales location, being the only treatment, which showed effect with respect to control treatment.

Table 6. Location * strain interaction for incidence and yield variables in the evaluation of four *Trichoderma* spp. strains in three locations of Nariño, department, Colombia

Incidence Location			
Strain	Gualmatán	Ipiales	Pupiales
Control	89.99 A	89.9 A	100 A
Chemical	89.99 A	76.35 B	91 AB
C21	87.68 A	88.04 A	89 AB
C12	85.78 A	83.89 B	80 B
Tukey comparator (α 0.05)	6.97	5.77	15.11
Green pod yield Location			
Strain	Gualmatán	Ipiales	Pupiales
C21	6077.3 A	824 A	16138.3 A
Chemical	3678.3 A	1782 A	14740 AB
C12	4887.0 A	997 A	14699.9 AB
Control	4393.0 A	820 A	13044 B
Tukey comparator (α 0.05)	2910.1	981.51	2316.8

Given these concerns, it is possible the pathogen incidence of presence or pathogen absence throughout root redness observation have not allowed an accurate indicator of two evaluated strains (C12 and C21) efficiency. In many plants, when pathogen was able to penetrate and show some degree of root redness, the action of *Trichoderma* spp., could affect it by colonization of the root area. Therefore, the plants slightly affected, produced new roots or simply fulfilled their productive cycle. Díaz *et al.* (2012), consider the levels incidence depend on the provenance of the terrain, variety traits, temperature and humidity. Although, this variable has no direct relation with some components of yield being necessary in future research to evaluate also the severity degree and its effect on the plant performance.

Field assay

Green pod yield

For Gualmatán and Ipiales locations, there were no differences between C21 and C12 strains, chemical control and control treatment. In Gualmatán location, yields ranged between 6077 and 3678 kg.ha⁻¹, while in Ipiales location, they were between 1782 and 820 kg.ha⁻¹. In Pupiales location, it was observed that C21 treatment with an average of 16138 kg.ha⁻¹, had differences with the control treatment, which obtained 13044 kg.ha⁻¹. The chemical treatments and C12 with 14740 and 14699 kg.ha⁻¹, showed no differences with respect to C21 and chemical control (Table 6). This information is useful to argue that all treatments were equally affected by the pathogen.

Therefore, it was not possible to demonstrate the efficiency of C21 and C12 *Trichoderma* strains in the antagonism on *Fusarium oxysporum* f. sp. *pisi*. In Ipiales location, the lowest yields were obtained from the three evaluated locations, which did not exceed 1800 kg.ha⁻¹. The lower incidence of *Fusarium oxysporum* f.sp. *pisi*., observed in C12 and chemical control on C21 and control treatment, was not reflected in the yield, where all the treatments had low averages without statistical differences among them. These results suggest, the pathogen in this location was highly aggressive and in addition, to being present in the plant roots with incidence values between 76.35 and 89.9%, could severely affect the xylem vascular bundles, which caused its obstruction reducing the nutrient passage and decreasing performance for all treatments. In Pupiales location, yield values in four treatments, are relatively high (Table 6). However, the pathogen was able to enter into the plant roots and marked a high incidence (number of plants with some degree of root redness), suggesting there was no severe affectation of the vascular bundles. In this location, C21 strain, outperformed the control treatment. However, in the incidence measurement, the average of these two treatments did not show differences, which indicates in the treatment with the C21 *Trichoderma* strain, if the pathogen *F. oxysporum* entered into the roots but did not colonize them enough to affect yields. Pineda & Tortolero (1995), indicates the pathogen biological control with antagonists potential is a long-term process that must be implemented with the usual crop management.

Conclusion

in vitro conditions, the C2, C7, C12 and C21 *Trichoderma* strains had high antagonistic capacity on *Fusarium oxysporum* f. sp. *pisi*.

In greenhouse conditions, C12 and C21 *Trichoderma* strains showed the greatest antagonistic effect against *Fusarium oxysporum* f. sp. *pisi*.

In field conditions, the strains effect was differential. In Gualmatán location, C12 and C21 strains, showed no antagonistic capacity. In Ipiales and Pupiales locations, the C12 strain had lower incidence of *F. oxysporum*, than the control treatment on the yields. In Pupiales location, C21 strain outperformed the control treatment, even though the two treatments had a similar incidence.

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